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Art. IX.—*Studies in the Physiology of Host-Parasite Relations.*

1. The Effect of *Bacterium solanacearum* on the Water Relations of Plants.

By B. J. GRIEVE.

Botany School, University of Melbourne.

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I. Introductory.

It is proposed in this series of papers to examine selected pathological conditions in plants brought about by parasitic infection, in an endeavour to obtain a deeper insight into the physiology of host-parasite relations, with special reference to stimulation effects and wilting. For the purpose two xylem parasites, *Bacterium solanacearum* and *Fusarium lycopersici,* a phloem parasite *Aplanobacter Michiganense,* and a virus Spotted Wilt of tomato, have been selected for study. The present paper is devoted to an examination of the effect of invasion by *Bacterium solanacearum* on the water relations of host plants.

Plants infected by this organism frequently show as first symptoms epinasty of leaves, and the production of adventitious roots on the stem. Leaf epinasty is followed by unilateral flaccidity of leaflets, and finally the plant shows severe wilting. The march of transpiration and of absorption was determined during these successive phases, and correlated with the degree of invasion. The theories put forward by other workers and the belief that the wilting effect may be due to—(*a*) gum formation, (*b*) tylose formation, (*c*) toxins, (*d*) mechanical blocking, are examined in the light of these and other experimental results obtained.

II. Experimental Methods.

1. *Material:*—Tomato plants (variety Marglobe) and potato plants (variety Carman) were used as experimental material. A pure culture of *B. solanacearum* isolated from potato tubers served as the source of inoculum.

2. *The Measurement of Absorption and Transpiration:*—Weighing potometers were used for the determination of absorption and transpiration in healthy and infected plants. For experiments extending over one to two weeks (using tomato plants 8 to 9 inches in height), a tower type of potometer with vertical side-arm absorption tube was found suitable, while a small flask potometer fitted with a horizontal absorption tube served for short-term experiments using smaller plants.

Weighings of the tower type potometers were made on a transpiration balance and of the smaller flask type on a precision balance. Absorption values were obtained in each case from the calibrated absorption tubes. To minimize errors due to hydrostatic pressure effects in the vertical absorption tubes of the larger potometers, the meniscus movement was limited to 3 cm. before readjusting. Corrections were also made for volume variations due to temperature changes. Experimental plants were grown to a suitable size in washed sand watered with Shive's No. R5 C2 solution (1915) and after the roots had been washed free of sand the plants were transferred to the potometer. The method of removal of a plant from the sand in which it had been growing was to flood the container by placing it sideways in a larger container full of water. By gentle movements of the plant the roots were then disengaged from the sand without injury, and after further washings the plant was ready for insertion in the potometer. A short length of vaselined cotton wool was wound round the base of the stem which part was inserted into the appropriate slit in the rubber cork. The apparatus was then assembled, all air bubbles being eliminated. The potometer flask had previously been filled with boiled and cooled Shive's solution. Luting wax was used to give a watertight seal. After setting up, the plant was left for several hours, generally overnight, to recover from any "transference reaction." Algal growth in the culture solutions was inhibited by the use of brown paper shields.

The nutrient solution was maintained in each container by means of a reservoir connected by a tap and the solution itself was changed every third day in the case of tower potometers and each day in the case of the flask potometers. In long term tests the growth of the experimental plants was quite comparable to that of plants which were kept growing in sand culture.

For the determination of transpiration alone, plants were grown in metal or glazed containers, and when ready for use rubber covers were fixed in position. Loss in weight over specified periods was taken as a measure of the transpiration. Water was added daily to bring the containers back to their original weights. Evaporation and temperature records were kept during experiments. The plants selected for experimentation were of similar size and equal vigour. For comparative experiments they were grouped in pairs of closely similar size, and the containers were so arranged on the greenhouse bench that all were exposed to approximately the same environmental conditions.

For comparing the rates of water loss from intact leaves and leaflets of healthy and infected plants, Livingstone and Shreve's cobalt chloride method (1916) was followed, while a delicate torsion balance was utilized for comparing the rates of water loss

from detached leaflets of healthy and invaded plants. The procedure using the torsion balance was to cut off the companion leaflets, vaseline the cut ends, then weigh each as rapidly as possible. The leaflets were then suspended in still air, weighings being at hourly intervals for three hours.

3. *Leaf Area Determinations:*—When leaf areas were obtained at the close of an experiment the blue print and planimeter method was used. For daily leaf area determinations in tomato and potato, the special methods developed by Porter (1937) and Clements and Goldsmith (1924) were followed. When an experiment was carried to the wilting phase of disease the turgidity of wilted leaflets was restored by floating them on water for half an hour.

4. *Inoculation Technique:*—The organism was introduced by prick inoculation into the xylem of stem or root of the test plants as specified in the text. Control plants were pricked with a sterile needle.

5. *Histological Technique:*—To facilitate estimation of interference with water movement in infected plants, the procedure was adopted of cutting off the tips of roots at the end of an experiment and placing the plants in eosin solution for half an hour. Sections for microscopical examination were then cut at the bases of all petioles and at different root and stem levels. Material for paraffin sectioning was fixed in 70 per cent. alcohol and nitric acid. This prevented the diffusion of the bacteria from the vessels. Rawlins' modification of Stoughton's method (1933) was frequently employed for staining.

III. The March of Transpiration in Relation to the March of Invasion.

To obtain as complete a picture as possible of the influence of bacterial invasion on the transpiration relations of inoculated plants, experiments were carried out under three sets of environmental conditions which markedly affected the speed of invasion and the reaction of the host plant to it. These were as follows:—

(*a*) In a glasshouse during summer months (rapid invasion).
(*b*) Under deep shade conditions in a glasshouse during summer months (moderately rapid invasion).
(*c*) In a glasshouse during autumn months (slow invasion).

(*a*) Transpiration Relations when Invasion is Rapid.

Experiment 1.—Eight healthy tomato plants 9 to 10 inches in height were selected and grouped in pairs. The members of each pair were very similar in height and bore the same number of leaves. Transpiration was recorded during a preliminary two-day period, after which one plant of each pair was prick inoculated

in one bundle near the base of the stem. Transpiration records were continued until the inoculated plants were wilting. The results of the experiment are embodied in Table I. and text figs. 1.

TABLE I.—PROGRESS OF INVASION AS INDICATED BY SYMPTOMS. EXPT. I.

EXPT. PLANT.	TIME IN DAYS AFTER INOCULATION.					
	1	2	3	4	5	6
1 (8 leaves)	—	—	2 leaves showing unilateral wilting	3 leaves wilting; 3 more wilting unilaterally	6 leaves wilting; 1 leaf unilaterally wilted; 1 leaf flaccid	All 8 leaves wilting
3 (9 leaves)	—	—	—	1 leaf wilting; 2 leaves showing unilateral wilt; 1 leaf flaccid	4 leaves wilting; 2 leaves showing unilateral wilting	7 leaves wilting; 2 leaves showing unilateral wilting
5 (9 leaves)	—	—	—	—	2 leaves showing unilateral wilting	1 leaf wilting; 2 leaves showing unilateral wilting
7 (11 leaves)	—	—	—	2 leaflets on 1 leaf flaccid	2 leaves wilting unilaterally	2 leaves wilting; 2 leaves wilting unilaterally

2, and 3. During the first two days after inoculation the transpiration rate of the four inoculated plants increased relative to the controls but when tested statistically using the "t" test this increase was not found to be significant. From the fourth

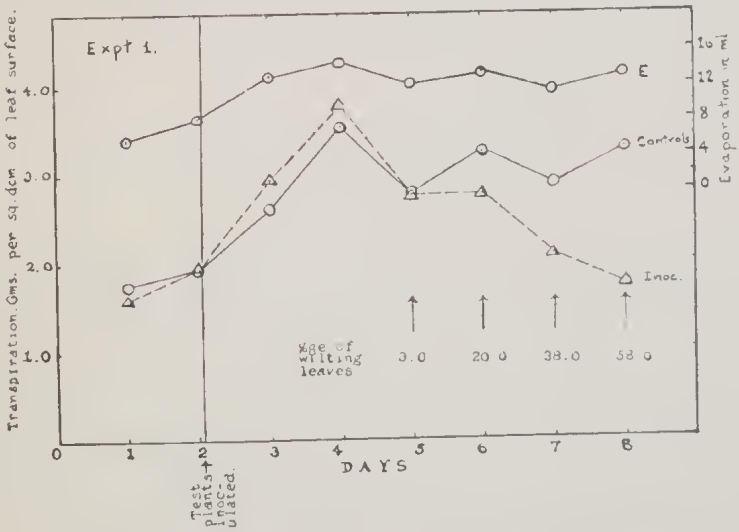

FIG. 1.—The march of transpiration in relation to the march of invasion when invasion is rapid. Expt. 1. Mean values for four control and four inoculated plants.

day after inoculation a continuous decrease in the rate of the inoculated plants occurred but the difference barely became significant as determined by the "t" test, even by the seventh day after inoculation when the experiment was terminated. In this connexion it may be stated that in examining statistically the averaged results for the four test and control plants, a complicating factor is introduced by the differential speed of bacterial invasion in each inoculated plant. Thus in test plants 1 and 3, by the sixth day after inoculation most leaves were wilting and transpiration was reduced greatly below that of the controls (see text fig. 2). In test plants 5 and 7 on the other hand symptoms

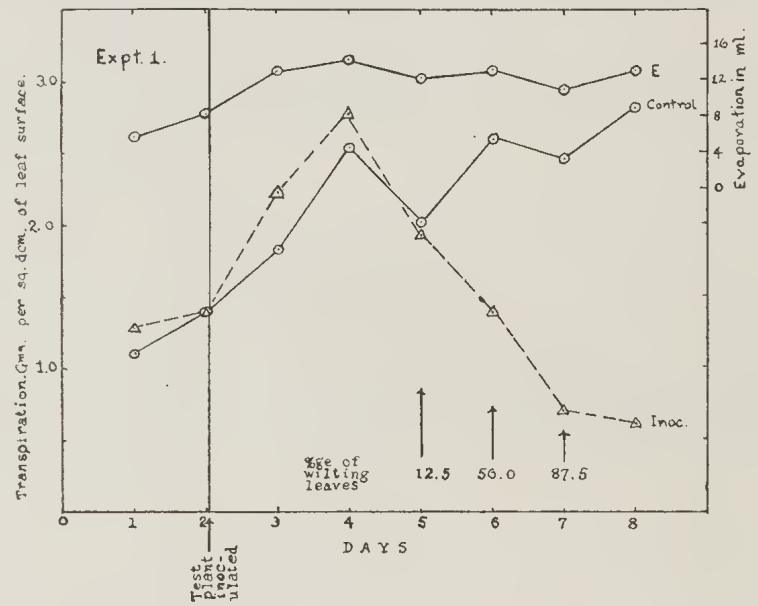

Fig. 2.—The march of transpiration in relation to the march of invasion when invasion is rapid. Expt. 1. Test plant 1 and Control.

developed later and reduction in transpiration was only commencing to show by the sixth day. The experiment would have had to be continued to the tenth or twelfth day for them to reach a comparable stage of wilting to that of test plants 1 and 3. This time lag reduces the differences between test plant and control series when averaged results from day to day are taken and examined by the "t" test. The results in individual cases in this experiment, and in others to be described, leave no doubt about the actual depression of the transpiration rate of the infected plants relative to the controls.

An interesting point is that little difference between the transpiration rates of the inoculated and control plants developed

until one or two leaves (out of a total of 7 to 9 on each plant) were showing some degree of wilting. This result suggested that in the early stages of wilting, increasing invasion of vessels was accompanied by increased conduction in vessels still free, while non-affected leaves connected with these transpired an increased volume of water. Other evidence on this point is presented later.

When the mean cumulative water loss by transpiration from test plants 1 and 3 and from their controls is considered (text fig. 3), it is seen that a definite reduction in the total volume of

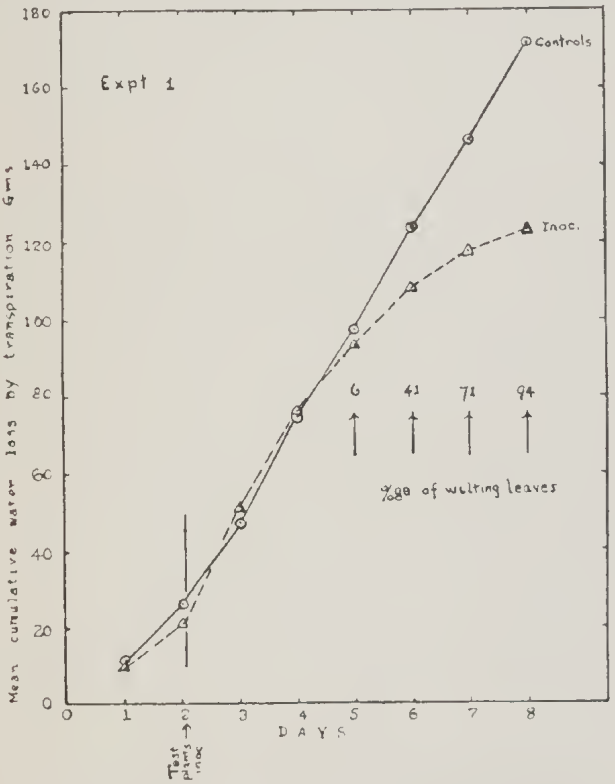

FIG. 3.—Comparison of the mean cumulative transpiration losses from two healthy and two inoculated tomato plants. Expt. 1.

water transpired relative to the controls is becoming apparent by the fourth day after inoculation when 41 per cent. of the leaves were showing degrees of wilting. The gradual nature of the interference with transpiration is clearly shown in this graph.

In a second experiment in which a set of four inoculated and four healthy plants were used, results compatible with Experiment 1 were obtained.

(*b*) Transpiration Relations when Invasion is Moderately Rapid.

Experiment 3.—Plants were placed for the period of the experiment under dense shade conditions in a glasshouse with the object of reducing the speed of invasion and favouring the induction of epinasty. Temperature remained optimum for invasion. Epinastic response occurred in a high percentage of leaves of all the infected plants and it was found in this experiment that their transpiration rate began to show reduction one day after the development of the epinasty. This reduction became more pronounced each succeeding day that the condition persisted. Owing to the low transpiration rate under the shade conditions of the experiment, leaves remained turgid, though reflexed, until a high degree of invasion was reached.

(*c*) Transpiration Relations when Invasion is Slow.

Experiment 4.—This experiment was carried out in autumn and early winter and was designed to give information on the effect of slow invasion on transpiration. Temperature and light conditions were such as to allow fairly rapid growth of the test plants, but were sub-optimal for bacterial growth. Ten tomato plants were selected, grouped in pairs of closely similar size and their daily transpiration losses over a ten-day standardization period determined. One plant of each pair was then prick inoculated with *Bacterium solanacearum* in a vascular bundle near soil level. Transpiration losses of each grouped pair and the progress of invasion as indicated by epinastic response of leaves (Pl. XI., fig. 1), unilateral wilting and bilateral wilting of leaves were recorded during 30 days when the experiment was terminated. Results are expressed in Table II. and text figs. 4 and 5. For convenience of presentation the mean transpiration losses for each set of plants were grouped in five-day runs. Depression of the rate from infected plants relative to the controls began to be apparent by the tenth day after inoculation. This difference increased as invasion continued up to the close of the experiment when a high percentage of wilting leaves was showing in the infected plants. At the close of the experiment the difference in rates between inoculated and healthy plants was significant at the 1 per cent. level, using the "t" test. If one considers any given pair of the inoculated-control series a clearer picture is given of the effect of slow invasion on transpiration. Results for test plant 3 and its control (see Table II.) are given in text fig. 5. During the ten-day standardization period there is close agreement between the volumes of water transpired by each plant and this relation continues for a further twelve days after test plant 3 had been inoculated. By this time symptoms were commencing to develop. It was not, however, until four out of the eight leaves on the plant were showing epinastic response

that its transpiration began to drop below that of its control. It is to be noted that the water loss from the infected plant remained relatively high up to the close of the experiment, the loss from the healthy plant not being overwhelmingly greater except on days when the climatic conditions favoured high transpiration. The maintenance of this relatively high rate is believed, on the basis of eosin and section tests, to be due to the failure of the bacteria to penetrate in sufficient numbers into the freshly developing xylem vessels in the plant, so that a conducting channel between the roots and the apical leaves was kept open.

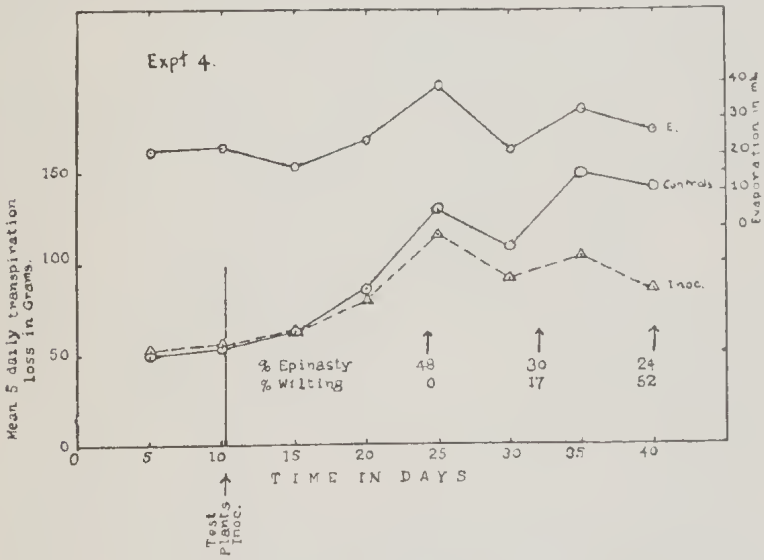

FIG. 4.—The march of transpiration in relation to the march of invasion when invasion is slow. Comparison of the five daily average transpiration from healthy and inoculated tomato plants. Expt. 4.

Results of this experiment parallel those where invasion was rapid, differing only in the prolongation of the epinasty phase and in the strong development of secondary tissues. The prolongation of the epinastic phase in the presence of increasing numbers of bacteria has an important bearing on the "bacterial" theory of wilting and will be referred to later.

To summarize the results obtained from the four experiments in this section we may say that each has shown that under varied environmental conditions—(*a*) the march of transpiration in infected plants runs parallel with that in control plants for one to two days after the appearance of well defined symptoms in the former. The depression in the transpiration rate which then becomes evident does not appear to be directly conditioned by available leaf area; (*b*) the reduction of the transpiration rate in infected plants under all conditions of invasion is a gradual process and no sudden disturbance of this function occurs.

TABLE II.—PROGRESS OF INVASION AS INDICATED BY SYMPTOMS. EXPT. 4.

EXPT. PLANT.	TIME IN DAYS AFTER INOCULATION.					
	12	15	20	22	25	29
1 (9 leaves)	—	4 leaves showing epinasty	One leaflet on 1 epinastic leaf wilting, otherwise no change	5 leaves showing epinasty; leaflets on 2 epinastic leaves wilting	3 epinastic leaves showing unilateral wilting; 2 unchanged	4 leaves severely wilted; 3 leaves wilting; 1 epinastic; 1 normal
2 (9 leaves)	1 leaf showing epinasty	5 leaves showing epinasty	One leaflet on 1 epinastic leaf wilting, otherwise no change	Unilateral wilting in 3 epinastic leaves	No change	No change; 5 leaves normal
3 (8 leaves)	1 leaf showing epinasty	4 leaves showing epinasty	Unilateral wilting in 1 epinastic leaf; rest unchanged	Unilateral wilting in 3 epinastic leaves	No change	3 leaves wilted 3 epinastic; 2 normal
4 (10 leaves)	—	3 leaves showing epinasty	No change in epinastic leaf; 1 leaf wilting unilaterally	4 leaves showing epinasty; 1 leaf wilting unilaterally	No change	3 leaves wilted; 1 unilaterally wilted; 4 epinastic, 2 normal
5 (10 leaves)	1 leaf showing epinasty	6 leaves showing epinasty	No change	Unilateral wilting in 3 epinastic leaves	Unilateral wilting in 5 epinastic leaves	8 leaves wilting; 1 flaccid; 1 normal

COMPARISON OF HOURLY TRANSPIRATION RATES OF CONTROL AND INFECTED PLANTS AT THE INCIPIENT WILTING STAGE.

During the two days in which wilt symptoms were developing in test plants 1 and 3 of Experiment 1, a comparison of their hourly transpiration rates with those of their controls was made (text fig. 6). Both inoculated plants appeared fresh and normal at 8 a.m. when the hourly observations were commenced. By 12 noon two leaves of test plant 1 were wilting and one was flaccid, while in test plant 3 one leaf was wilting and two leaves were showing commencement of unilateral wilting. By 2 p.m. the picture was much as set out in Table 1, column 4. Turning to the transpiration record (text fig. 6) it may be observed that on the first day the transpiration rates of the control and inoculated plants rose by almost equal increments of rate until 11 a.m. During the period 11 a.m. to 1 p.m. the controls transpired at a considerably higher rate. It was during this period that flaccidity was becoming evident in certain leaves of the inoculated plants. Between 1 and 2 p.m. the transpiration of inoculated plants showed a greater increase relative to the controls, but between 2 and 3 p.m. with evaporation still increasing, the inoculated plants showed a decrease of 27 per cent. relative to the preceding hour's rate, while the transpiration rate of the

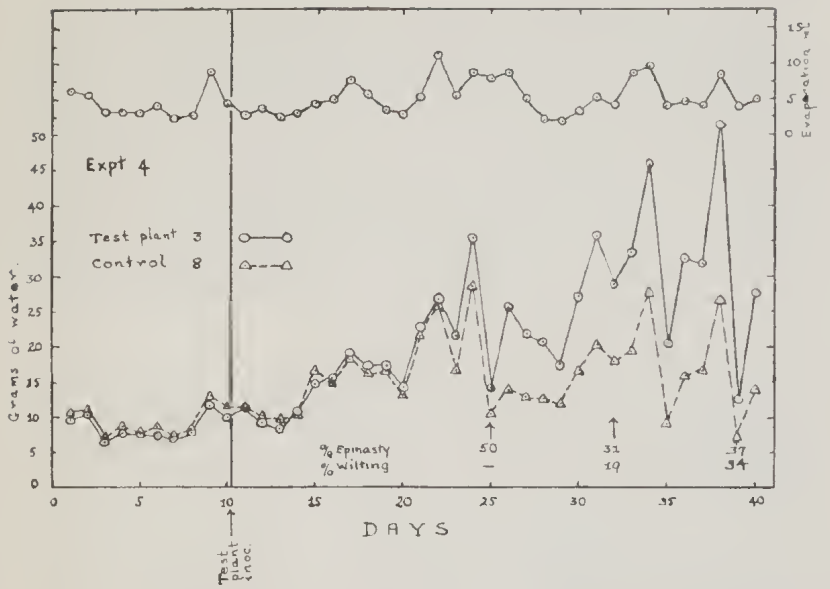

FIG. 5.—Comparison of transpiration from a healthy and an inoculated plant under conditions of slow invasion. Expt. 4. Test plant 3 and Control.

controls continued to rise. From 3 to 4 p.m. the rates of transpiration in both sets were decreasing, but the relative decrease was 10 per cent. greater in the controls. In the record for the following day the only noteworthy feature was a repetition of the rate increase of the infected plants relative to the controls (22 per cent. : 2.5 per cent.) during the 1 to 2 p.m. period. A possible explanation of these effects is that with the commencement

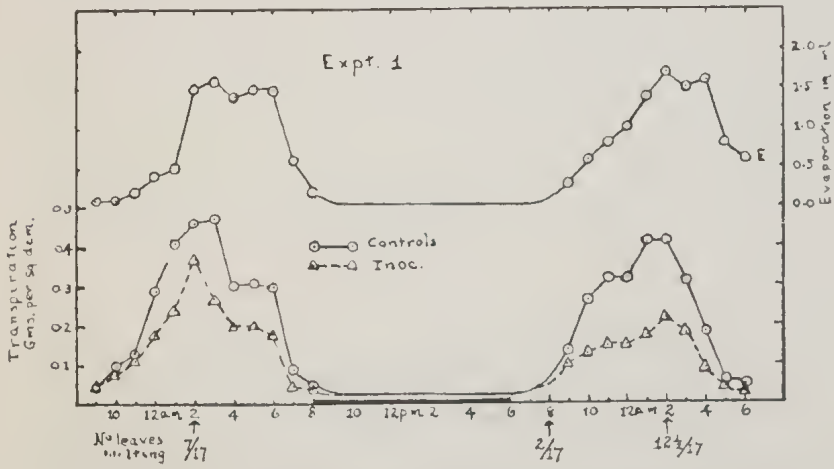

FIG. 6.—The daily march of transpiration in healthy and inoculated plants when bacterial wilting is just commencing. Mean values for Test plants 1 and 3 and their Controls. Expt. 1.

of bacterial wilting the stomata open more widely and the transpiration rate rises steeply for a short time after the manner described by Knight (1922) for plants wilting from lack of water. It is suggested that the subsequent earlier fall in the transpiration rate of infected plants may be due to the fact that a transpiration-limiting water content of the mesophyll cells is reached more rapidly in the infected plants than in the controls owing to the operation of two factors:—(*a*) The more widely open stomata during the preceding phase, and (*b*) the interference with the conduction of water to the leaves. Further investigation of these effects is being made with special reference to stomatal movements during the wilting phase.

The graph also shows that both in healthy and infected plants the hourly march of transpiration follows the normal course observed for many herbaceous plant types. In the healthy plants it is evident that the rate of transpiration increased more rapidly than the rate of evaporation, as registered by the atmometer, until a maximum was reached and the later increase in the exaporating power of the air was not reflected in the rate of water loss. The curves also show that transpiration increases rapidly during the daylight hours when the stomata are open and falls very low during the night hours when the stomata are closed. The overnight restoration of turgidity in wilting leaflets of infected plants is due to this decrease in transpiration.

Observations on Temporary Overnight Recovery of Turgidity in Flaccid Leaves.

During the course of the above experiments it was observed that leaflets of plants which become flaccid or wilted during the day often recovered their turgidity during the night. This recovery from flaccidity after a second day was frequently repeated during a second night, but usually by the third day the wilting became irreversible. Typical examples of this temporary recovery in infected plants are recorded in Table III. This recovery is of course due to the fact that the water content of the flaccid or wilted leaves builds up overnight while transpiration is reduced, but the effect is important in relation to certain theories advanced on the cause of bacterial wilting and the bearing of these observations on this wilting effect will be considered in Section VII.

IV. Comparison of the Rates of Transpiration from Companion Leaflets during Epinasty.

The epinastic response of leaves of infected plants, which has been referred to in connexion with Experiments 1 to 4, has been shown in an earlier paper (Grieve, 1939) to be conditioned by the path of invasion and generally to be associated with the

presence of bacteria at the base of the petiole. Depending on the speed of invasion, leaf epinasty is followed sooner or later by unilateral wilting of leaflets. Data presented in Section III. showed that transpiration began to decline one or more days after the development of epinasty and usually not until one or two

TABLE III.—OBSERVATIONS OF TEMPORARY OVERNIGHT RECOVERY OF TURGIDITY IN WILTING LEAVES OF INOCULATED PLANTS. DATA FROM EXPT. 3. LETTERS DENOTE LEAVES; *a* = LOWEST ON STEM.

		Days after Inoculation.			
		4	5	6	7
Test Plant No. 3. (8 leaves)	9 a.m.	Leaves *b*, *c* and *d* showing epinasty	Leaflets of *d* turgid	All leaves turgid	Leaves *d* and *e* turgid, leaf *g* unilaterally flaccid, leaf *b* unilaterally wilted
	11 a.m.	No change	Unilateral wilting in leaf *d*	Unilateral wilt-developing in leaves *b*, *d* and *g*	
	1 p.m.	Unilateral wilting in leaf *d*	No change	Unilateral wilt in leaves *b*, *d*, *e* and *g*	
	3–5 p.m.	No change	Unilateral wilting in leaves *b*, *d*, and *g*.	No change	Leaves *b* and *d* wilting bilaterally; leaves *e* and *g* unilaterally
	7 p.m.	Leaflets of *d* almost turgid	Leaflets of *d* almost turgid; leaflets of *b* and *g* still flaccid	No indication of recovery	No recovery
Test Plant No. 4. (9 leaves)	9 a.m.	Leaf *b* showing epinasty	No change from preceding day	All leaves turgid	Leaves turgid except *b* which remained flaccid
	3 p.m.	No change	Unilateral wilting commencing in leaves *b*, *e* and *g*	Unilateral wilting in leaves *b*, *e* and *g*	Leaves *b* and *e* wilting bilaterally; leaves *c* and *d* showing epinasty, while *f* and *g* show unilateral wilting
	7 p.m.	No change	All leaves recovered turgidity	No change	No change

leaves of a plant were wilting. It therefore appeared desirable to compare transpiration rate in companion leaflets of leaves showing epinasty or unilateral wilting and to relate the results obtained to transpiration relations discussed in the preceding section. At the outset, determinations were made on companion leaflets of healthy tomato and potato plants in order to find the degree of variation in transpiration rate between such leaflets.

Experiments on such companion leaflets using both the "three colour strip" method and the torsion balance method showed that the differences in rate between them were comparatively small (Table IV.). Determinations were next made on companion leaflets of leaves showing epinasty. The results (Table IV.) were examined statistically by the "t" test and showed that at the 1 per cent. level there was a significantly lower transpiration rate from one companion leaflet of a pair in plants showing leaf epinasty. Sections and eosin test showed the side with the reduced rate to be the invaded side. These experiments made it plain that some increase in transpiration rate must have occurred either in other leaves on the plant or in non-affected companion leaflets in a leaf showing epinasty, to account for the maintenance of transpiration rate which has been recorded in Expts. 1 to 3. It was not found practicable, however, to ascertain by the above methods whether part of this compensatory increase took place in the non-invaded side.

TABLE IV.—COMPARISON OF THE RATES OF WATER LOSS FROM COMPANION LEAFLETS (A, B) OF HEALTHY PLANTS AND FROM COMPANION LEAFLETS OF INFECTED PLANTS SHOWING LEAF EPINASTY. (IN INFECTED PLANTS A = INVADED SIDE.)

(*a*) *Torsion Balance Experiments.*
Water loss in mgms. per sq. cm. per hour.

Tomato.				Potato.			
Healthy.		Infected.		Healthy.		Infected.	
1A ..	0·56	1A ..	0·22	1A ..	1·01	1A ..	0·25
1B ..	0·49	1B ..	0·80	1B ..	0·96	1B ..	0·59
2A ..	0·40	2A ..	0·48	2A ..	0·83	2A ..	0·29
2B ..	0·39	2B ..	0·47	2B ..	0·83	2B ..	0·41
3A ..	0·30	3A ..	0·21	3A ..	1·08	3A ..	0·96
3B ..	0·24	3B ..	0·58	3B ..	1·08	3B ..	0·86
4A ..	0·41	4A ..	0·36	4A ..	0·28	4A ..	1·84
4B ..	0·55	4B ..	0·69	4B ..	0·31	4B ..	2·23
5A ..	1·26	5A ..	0·37	5A ..	0·47	5A ..	0·99
5B ..	1·14	5B ..	0·53	5B ..	0·38	5B ..	1·09
6A ..	1·60	6A ..	0·33	6A ..	0·25	6A ..	0·15
6B ..	1·60	6B ..	0·50	6B ..	0·23	6B ..	0·40
7A ..	0·17	7A ..	0·17	7A ..	0·25	7A ..	0·12
7B ..	0·18	7B ..	0·82	7B ..	0·35	7B ..	0·26
8A ..	1·78	8A ..	1·12	8A ..	0·27	8A ..	0·72
8B ..	1·77	8B ..	1·00	8B ..	0·26	8B ..	0·58
9A ..	0·81	9A ..	0·40	9A ..	0·11	9A ..	1·41
9B ..	0·69	9B ..	0·63	9B ..	0·13	9B ..	2·90
10A ..	0·31	10A ..	0·20	10A ..	0·25	10A ..	0·17
10B ..	0·23	10B ..	0·30	10B ..	0·23	10B ..	0·30

TABLE IV.—*continued.*
(*b*) *Cobalt chloride method.*
POTATO.

Healthy.				Infected.					
1A	40	seconds		1A	150	seconds	6A	180	seconds
1B	40	,,		1B	55	,,	6B	60	,,
2A	60	,,		2A	180	,,	7A	115	,,
2B	55	,,		2B	55	,,	7B	30	,,
3A	45	,,		3A	105	,,	8A	145	,,
3B	40	,,		3B	47	,,	8B	117	,,
4A	160	,,		4A	90	,,	9A	210	,,
4B	167	,,		4B	30	,,	9B	175	,,
5A	63	,,		5A	150	,,	10A	145	,,
5B	60	,,		5B	30	,,	10B	60	,,

V. The Effect of Invasion on Transpiration/Absorption Relations.

The wilting effect has so far been examined only in relation to water loss. Since theories of wilting in relation to bacterial invasion have postulated a breakdown of the absorption mechanism it was necessary to ascertain the effect of invasion on water uptake. In the following experiments absorption and also transpiration rates were recorded using potometer types A and B described under Methods. Initial experiments dealt with the relation between the rate of absorption and of transpiration in healthy tomato plants. The problem of water balance has received attention from various workers. Thus Montfort (1922) using the potometer, investigated the relationships between transpiration and absorption in *Zea mays* and *Impatiens parviflora* grown in Knop's solution and on transfer to peat and bog water. He found the transpiratory quotient (T/A) in Knop's solution and in peat and bog water to be generally greater than unity. Lachenmeier (1932) on the contrary found for *Veronica beccabunga* and *Myosotis palustris* that absorption exceeded transpiration both in light (artificial) and dark, or T/A was less than unity. Kramer (1937) recorded for sunflower plants growing in nutrient solution, that transpiration frequently exceeded absorption during the day, but that the positions were reversed during the night. The result of a typical experiment with healthy tomato plants is shown in text fig. 7. It is seen that under the conditions of these experiments in a warm glasshouse, transpiration generally exceeds absorption during the day while absorption is always greater than transpiration during the night. These results are in closest accord with those of Kramer who also worked under glasshouse conditions. It may be noted here, however, that in later experiments carried out under artificial light, results approximating to Lachenmeier's were obtained (see Section VI.). In the above experiments readings and weighings were taken at 9 a.m. and at 5 p.m. giving the comparison between absorption and

transpiration during the day and night periods, but for the longer experiments now to be described, in which the march of these two functions was followed in connexion with invasion, values were recorded for 24 hour periods, readings being taken at 9 a.m. each day. Each plant served as its own control, 24 hourly readings of both water uptake and water loss being taken for some days before inoculation and continued until epinasty and wilting effects were visible after inoculation. Root and apical stem inoculations were practised in different experiments in order to differentiate between the effect on absorption of the presence or local absence of bacteria.

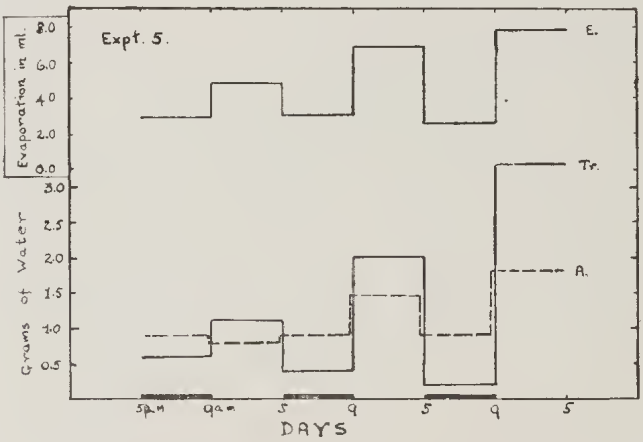

FIG. 7.—Rates of absorption and transpiration in healthy tomato plants during the day and night. Expt. 5.

(*a*) *Root Inoculation:*—Typical results are expressed in text figs. 8 and 9. No record of evaporation was kept for these experiments. The temperature record however, showed little variation and it has been observed in other experiments where both evaporation and temperature records were kept, that the curves followed roughly a parallel course. Consequently the falling transpiration and absorption rates shown in the later stages of the experiments were not due to changing environmental conditions but to the effect of the disease. Study of the text figures shows that there is no sudden interference with the absorption of water but that there is a gradual decrease in rate as the disease progresses. The plants were sectioned at various root and stem levels at the close of the experiments after being tested for eosin uptake through cut root tips. Examination then showed that the rate of absorption in the final stages was much higher than what might have been expected having regard to the number of vessels not conducting eosin. Thus in Experiment 9 (text fig. 8) for example, the absorption was 0.50 gms. over

24 hours (50 per cent. of its mean value up to inoculation) at a time when by eosin test it was shown that more than three-quarters of the total number of vessels of the main root were unable to conduct water. Similarly, conduction of water through the stem as judged by transpiration, was high in relation to the number of vessels still able to conduct as shown by eosin test. These observations link up with those of Section III.

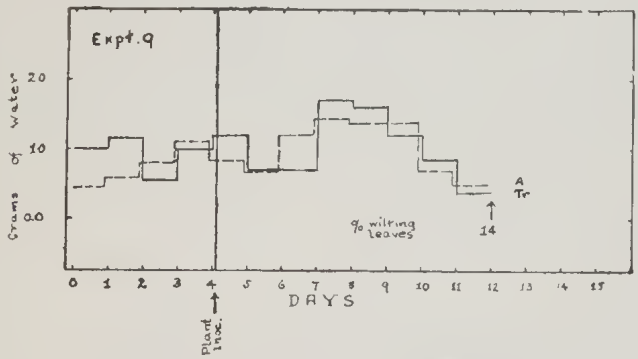

FIG. 8.—The effect of invasion by *B. solanacearum* on the transpiration and absorption of a tomato plant. Plant inoculated at base of stem. Expt. 9.

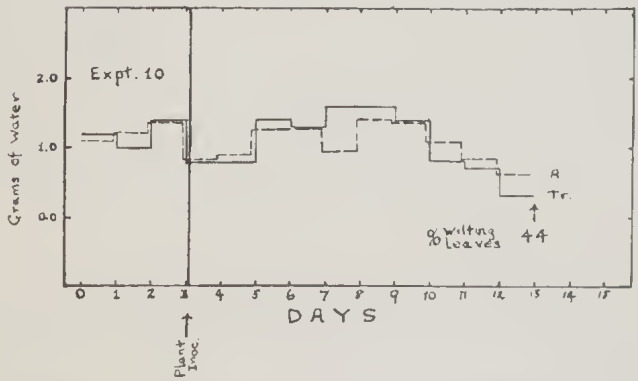

FIG. 9.—The effect of invasion by *B. solanacearum* on the transpiration and absorption of a tomato plant. Plant inoculated at base of stem. Expt. 10.

(*b*) *Apical Inoculation:*—This type of experiment was designed to determine whether any toxin or toxic substance produced by bacterial action could pass down to the roots and interfere with the absorption of water. A potato plant was used in one experiment, the inoculation point being high up on the stem. The experiment was carried on for four days by which time epinastic response but no wilting was showing in three apical leaves. The experiment was then stopped and an eosin and sectional analysis of the plant showed that the bacteria had grown

back down the vessels to the base of the stem but had not reached the roots. Just below the point where bacteria were present a certain amount of localised gum formation had occurred in and around vessels. Examination of the absorption rate (text fig. 10) shows that no depressant effect was exercised on the absorptive power of the roots by the bacteria growing back through the stem vessels. The results of this and other apical inoculation experiments taken in conjunction with the results for root inoculation experiments described earlier, indicate that the cause of wilting in tomato and potato plants is other than protoplasmic intoxication of root cells leading to reduced osmotic pressure and decreased absorption as was suggested by Hutchinson (1913).

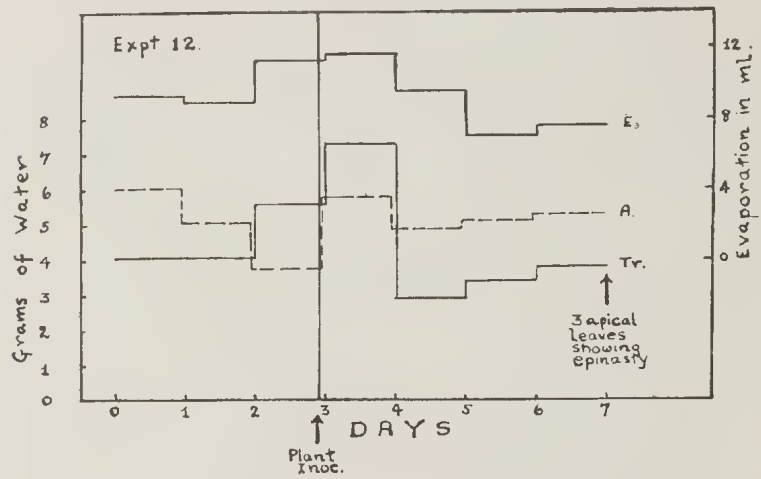

Fig. 10.—The effect of apical stem inoculation by *B. solanacearum* on transpiration and absorption in a potato plant. Expt. 12.

VI. The Effect of Artificially Reducing Leaf Area on Transpiration/Absorption Relations.

Results presented in Section III. indicated the development of a compensatory higher transpiration rate from unaffected leaves on infected plants to offset the reduction in rate in wilting leaves. To find whether this effect was the normal reaction of healthy plants on the reduction of their available leaf area the following experiments were performed. Transpiration/absorption rates in healthy tomato plants were determined using suitable potometers and then half or whole leaves were vaselined and the effect of this reduction in effective leaf area on the transpiration rate determined. Flask type potometers were used and the work was carried out in an insulated room, the illumination being provided by a 500 watt lamp. The tomato plants used were between five to seven inches in height, having six to eight well-expanded leaves. They had been grown in containers filled with washed sand and

watered with Shive's solution. After setting up in the potometer, using the procedure described under Methods, the plant was left for several hours, usually overnight, in the insulated room before an experiment was commenced. Further details of experimental technique with the small flask potometers may be given. The total weight of the potometer, nutrient solution and plant was approximately 180 grams. The balance used could carry up to 600 grams load and was sensitive to 8 mgms. The absorption was read off on a millimetre scale pasted along the absorption tube, and, after a correction for volume changes due to slight unavoidable rises in temperature had been applied, could be recorded in grams as the absorption tube in each potometer was carefully calibrated, using mercury. Experiments were first carried out to ascertain the normal absorption/transpiration relations in tomato plants under the conditions of these experiments. It was found that absorption tended to exceed transpiration slightly both in light and darkness. These results agree with those obtained by Lachenmeier for *Veronica beccabunga* and *Myosotis palustris* under similar experimental conditions. They differ from the results obtained under glasshouse conditions (Section V.) where absorption was found mainly to exceed transpiration only during the night. Absorption/transpiration values were next obtained at half-hourly intervals for a period of two to three hours, after which one half leaf or a complete leaf was vaselined on both surfaces. After vaselining a leaf in any experiment the plant was left for an hour before readings were resumed. Typical results are presented in text figs. 11 and 12. Vaselining of one leaf (fifth from base—nine leaves on plant) in Expt. 17 (text fig. 11) reduced the total leaf area by 18.2 per cent. and yet no reduction in the rate

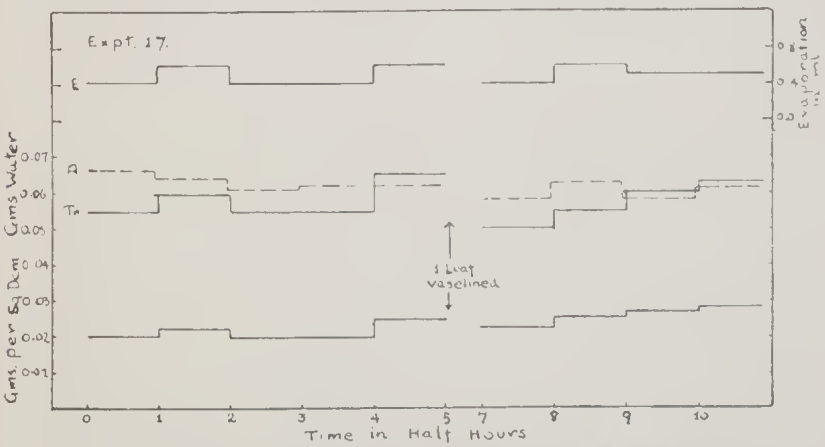

FIG. 11. Effect of vaselining one leaf on transpiration and absorption in tomato. Total number of leaves on the experimental plant was nine, and the fifth leaf from the base was vaselined. Original leaf area was 2.68 sq. dcm. and 2.19 sq. dcm. after one leaf was vaselined. Expt. 17.

occurred. In another experiment (No. 27) where one leaf (fourth from base—eight leaves on plant) was vaselined the reduction in leaf area was 28 per cent. and again no reduction in the rate of transpiration occurred. Text fig. 12 shows results obtained in two experiments when two and three half leaves respectively (cf., unilateral wilting in infected plants) were vaselined. The rate of transpiration under constant environmental conditions is more than maintained despite the reduction in leaf area. These results confirm the observations arising out of the

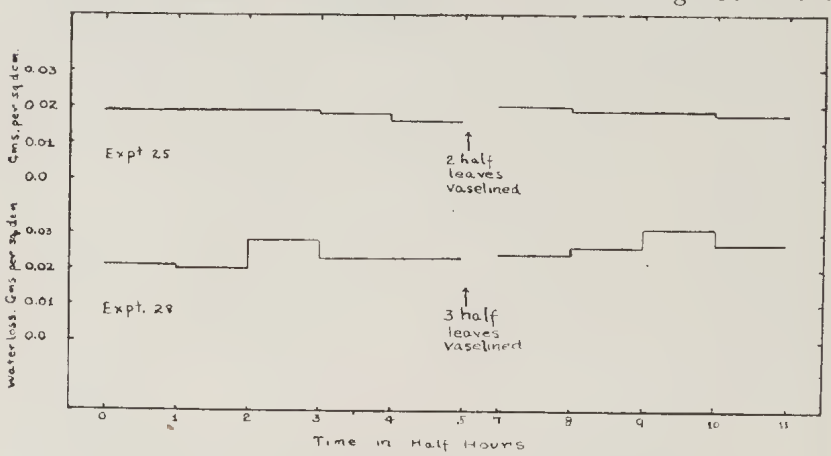

Fig. 12.—Effect of vaselining half leaves on the rate of transpiration. Expt. 25. Total number of leaves on the tomato plant was nine and half leaves of the 4th and 5th from the base were vaselined. Original leaf area was 3.66 sq. dcm.; residual area 2.89 sq. dcm. Expt. 28. Number of leaves six; half leaves of 2nd, 3rd and 4th from base vaselined. Original leaf area = 2.79 sq. dcm.; residual leaf area 1.89 sq. dcm.

study of transpiration rate in relation to bacterial invasion and wilting, in that they demonstrate that the rate of water loss may be maintained despite the reduction of available leaf area ranging up to 33 per cent. in the experiments so far reported. Since the experiments were carried out under controlled conditions of temperature and light it is clear that the supply of water to and the transpiration of water from the remaining leaves must have been increased. Reference to this effect occurs in Maximov's book *The Plant in Relation to Water* (p. 121, 2nd imp., 1935). He states "It has frequently been observed that if a portion of a plant is divided into separate pieces the intensity of transpiration is markedly increased. Famintsin (1883) in his book on the metabolism of plants cites the following experiment performed by his pupil Krutizky. A hawthorn shoot with eight leaves transpired 8 gm. of water a day; one with five leaves 5.2 gm.; and a shoot with only one leaf 4.9 gm. One the basis of the amount transpired in the third case, the first shoot should have transpired 39.2 gm. instead of 8 gm., and the second 24.5 gm. instead of 5.2 gm. This can only be explained by supposing that as leaves are successively removed from a shoot, the supply of water to the remaining still attached leaves is increased."

The chain of events involved in the increased supply of water to unaffected leaves in plants in which one or two leaves had been vaselined, may be visualized as follows, assuming the plant to be growing under conditions of constant light and temperature and to be well supplied with water. In the diagram (text fig. 13) A and B represent cells of two leaves which are served by vascular bundles which join at D, and C represents a cell in contact with the xylem of the root. We know that when a plant is transpiring

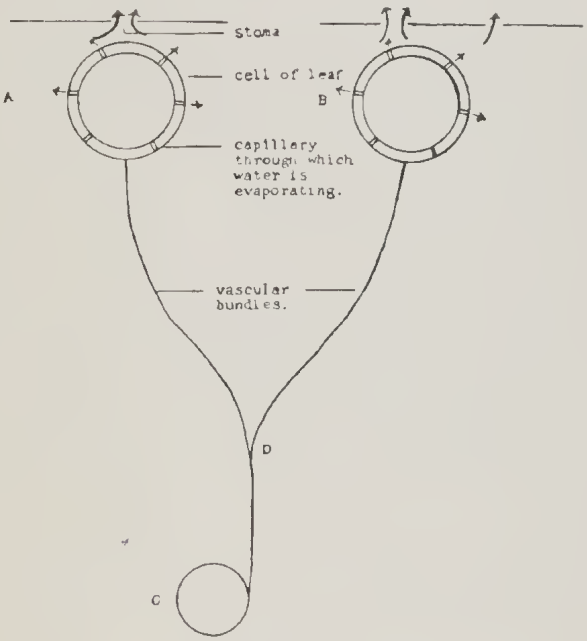

FIG. 13. Diagram to illustrate processes involved in increasing transpiration intensity with decreasing leaf area. See text.

a gradient of suction pressures is developed in the cells from the leaves down to the roots. The suction pressures developed at A and B may be assumed to be approximately equal and will be higher than the suction pressure at the point of water supply C. Consequently water will be drawn up through the stem vessels and approximately equal volumes will travel to cells A and B. The two leaves will be transpiring at rates proportional to the saturation deficit. Owing to the competition between them for water from C, however, the minute capillaries in the walls of the mesophyll cells A and B will not be completely filled with water and the outer layers of the cell walls will tend to dry. Under these conditions evaporation will be reduced. Now, if one leaf A be vaselined, exaporation is prevented and its cells show increasing turgidity. Applying the equation $S = P - T$, when S = suction

pressure, P = osmotic pressure, and T = wall pressure, it is clear that with increasing turgidity the suction pressure of cell A falls and finally the value of this approximates to the suction pressure of the water-supplying cell C, and no further water is drawn to cell A. Owing to the decreasing water demand from A, the suction pressure of cell C also falls as it becomes more turgid. This leads to the establishment of a greater suction pressure gradient between leaf cell B and the cell C. The net result is that a greater volume of water than before is drawn to cell B. As this cell shows increasing turgidity owing to the increased water supply, the water menisci in the minute capillaries in the walls of the mesophyll cells move outwards as the capillaries become filled with water, and finally there may even be liquid water present on the outside of the cell walls. Evaporation would then increase, occurring as from a free water surface in a vessel filled to the brim, and the gradient of suction pressure between B and C would be maintained in sufficient degree to cause the more rapid flow of water to B.

In infected plants where the bacteria are gradually filling vessels leading to one of two leaves, a somewhat similar chain of events may be postulated. The suction pressures of the cells of the leaves below which bacterial blocking is occurring, would of course rise, as less water passes to them, but as this force cannot be transmitted down to the point of water supply, the net result is that an increasing volume of water passes to the free leaf as the osmotic mechanism described above comes into play.

VII. Discussion of Results and their Bearing on the Physiology of Bacterially Induced Wilting.

It has been demonstrated in the preceding experiments that there is a progressive interference with the transpiration and absorption in infected plants as wilting of leaves increases. It is necessary now to interpret the experimental results obtained more closely in terms of the actual cause or causes of the disturbance of the water relations of the invaded plant. The history of investigations bearing on this point may here be considered.

Historical Survey:—Hunger (1901), from his examination of infected tomato and tobacco stems, concluded that the wilt was caused by the plugging of the vessels by the bacteria. He stated further that tyloses were abundant in the vessels of diseased tomato plants and believed that these structures, which apparently were induced by parasitic attack, helped to prevent the upward transport of water to the leaves. Hutchinson (1913), in a paper on Rangpur Tobacco Wilt caused by *B. solanacearum* in India, discounted the hypothesis that wilting was due to parasitic plugging of the vessels, in the case, at least, of tobacco wilt. From his observations on the disease and from experiments that he carried out, he was led to attribute the wilting effect to the action

of secreted toxins from the parasite on the cell protoplasm. His evidence may be summarized as follows:—1. Bacteria are not present in sufficiently large numbers in the vessels of wilted plants to cause any serious interference with the water supply of the plants. 2. A healthy tobacco plant may be cut half through the stem without causing wilt even in the leaves immediately above the cut. 3. An alcohol precipitate from a bouillon culture when dissolved in sterile water and fed into the vascular system of a healthy plant caused wilt in the course of a few days; when boiled it had no effect. He believed that the real explanation of wilting in relation to failure of water supply was that in the earlier stages of the disease at least, toxins produced by the organism caused protoplasmic intoxication leading to lowered osmotic pressure in the root system. In the later stages of the disease the water supply would be further interfered with by the formation and accumulation of gum masses in the vessels. Van der Meer (1929), who has done the most detailed work on this subject, carried out a number of experiments to ascertain the cause of wilting in infected tomato and tobacco plants and concluded that no one factor was responsible. The abridged account she gives in English of the results of her investigations on this point reads as follows:—" In which way will the bacteria influence the water supply in a plant? Possibly the co-operation of the factors mentioned in the different theories, results in a deficit of water. These factors may be: (1) root damage, (2) substances secreted by the parasite in the vessels, upon which the plant reacts by gum-formation and discolouration of the vessel walls, (3) accumulation of bacteria here and there in the vessels ".

The present investigation into the cause of wilting in the case of tomato and potato plants invaded by *B. solanacearum* will be considered under the following headings:—(1) gum formation, (2) tylose formation, (3) toxin production, and (4) mechanical blocking.

(1) *Gum Formation:*—Observations were made on the presence or absence of gum in the test tomato and potato plants used in the experiments described earlier in this paper and the possibility examined of this substance being concerned. In no case did the amount of gum present in the stem and petiolar vessels suggest the possibility of mechanical interference. Gum formation was in fact of much rarer occurrence in the vessels than in the cells surrounding the bundle groups. Gum formation was also more commonly observed in cortical cells and in cells of the interfascicular cambium than in the vessels. Its occurrence here was surprisingly localised. One transverse section of a stem or root would show gum in the cells while the next serial section would fail to show it. Longitudinal sections showed that the gum formation in the vessels was of sporadic occurrence and not in amounts sufficient to cause blockage. Experimental evidence, particularly from the absorption experiments, indicated that such

gum as was present in cells and vessels of the stem and root did not affect the passage of water. In experiments where apical inoculation was practised, gum appeared in root vessels and cells ahead of the bacteria which were multiplying and growing downwards in the xylem, and yet no obvious interference with the absorption of water occurred at that stage. Again eosin passed rapidly up through vessels in, or around which, gum formation was evident, but in which no bacteria were present. Van der Meer (1929) gives no data on the occurrence of gum in infected tomato plants, but apparently bases her conclusion that gum formation is involved in interference with water supply on experiments in which cut tomato seedlings were placed in the filtrate of beef-broth cultures of *B. solanacearum*. Her observations are as follows:—"After 12 hours there was no wilting to be observed, but cross sections of the tomato stems from the bacterial filtrate showed that the walls of some vessels were discoloured yellow and that some vessels contained gum." The occurrence of gum in some vessels after such treatment is not surprising, but the critical point is that no wilting was observed, so that her conclusion is regarded as being invalid in the case of tomato plants. Similar experiments to those above have been made by the author (Grieve, 1939), and again no wilting has been observed in the presence of gum formation; consequently for this reason and for the more cogent reasons adduced above, it is concluded in the case of tomato and potato plants invaded by *B. solanacearum* that gum formation in cells and vessels is not causally concerned with the interference with the water relations of such infected plants. The strain of *B. solanacearum* used would not infect tobacco, so that no attempt could be made to confirm the conclusions of Hutchinson and Van der Meer for this plant. There is, however, some evidence that the degree of gum formation and possibly its blocking effect is dependent on the host plant. Thus in *Impatiens balsamina* large amounts of gum have been observed in the vessels and it is hoped later to investigate its importance here.

(2) *Tylose Formation*:—The procedure adopted in investigating the incidence of tyloses was to cut transverse and longitudinal sections of the stems and roots, wash out the bacteria from the invaded vessels and then examine for the presence of these structures. Tylose formation does occur, but in only one isolated case where one bundle of a potato plant showed four to five vessels with well developed tyloses was there any real possibility of interference with water movement. In the vast majority of plants examined, the tyloses were small structures just showing as minute protuberances in relation to the size of the vessel at the time of onset of the wilting phase. The conclusion reached is that in tomato and potato plants tylose formation was not causally related to wilting and the disturbance of the water relations of the plant.

(3) *Toxin Production:*—Hutchinson's theory that wilting is due to toxins causing protoplasmic intoxication leading to lowered osmotic pressure is considered to be untenable, as far as the plants worked with by the author are concerned since (*a*) no confirmation of the presence of a toxin has been obtained on repeating Hutchinson's type experiment (Grieve, 1939); (*b*) absorption rate is not reduced when the bacteria are growing back from the top of the stem after apical inoculation until they are present in great numbers. If a toxin were involved and Hutchinson's view was correct, wilting due to lowered absorption should have occurred before the bacteria actually were present in the roots; (*c*) flaccid or even wilted leaflets of invaded plants recover overnight in the early stages of invasion (see Table III.), and (*d*) wilted leaves on being cut off and placed in water recover their turgidity. Such recovery could not occur in the continuous presence of a toxin. The example given under (*d*) indicated that the wilting effect is due simply to a temporary shortage of water. During the day water is lost more rapidly from the affected leaves than it can be made good through the partially invaded vessels. During the night reduced transpiration allows of building up of the water content of such leaves. The conclusions given here apply to tomato and potato and not to tobacco plants, but it is of interest to observe that Van der Meer applied the same criterion of recovery from wilting in discussing the possible occurrence of a toxin in invaded tobacco plants. She writes "If *B. solanacearum* secreted substances which poisoned the parenchymatic cells of the leaves, in my opinion a limp leaf had to persevere in its condition, when the vessels were experimentally enabled to transport water. . . . This observation suggests, that the inability of the xylem in the stem to transport sufficient water to the leaves has caused wilting, and contradicts the explanation of Hutchinson that the wilting symptoms of slime-disease would have been caused by protoplasmic intoxication and decreased osmotic pressure." In view of the result of this experiment and of others in which she was unable to confirm Hutchinson's results it is surprising to find she is unwilling to abandon the concept of toxin action in tomato and tobacco plants.

(4) *Mechanical Blocking:*—A considerable body of evidence regarding the part played by the bacteria in causing wilting by mechanical blocking, has been accumulated during the course of experiments extending over several seasons. At the close of all experiments when infected plants were showing epinasty or wilting, the procedure was adopted of cutting off the tips of their main roots and placing them in a solution of eosin for half an hour. Thin hand sections were then cut at the bases of petioles and stems, and roots were either sectioned at various levels or subjected to the maceration methods described elsewhere (Grieve, 1936). The results of some of these observations are embodied

in Tables V. and VI. which give the section analysis in the case of test plants used in certain of the transpiration experiments reported earlier in this paper. The picture for test plants 1 and 3 of Expt. 1 (Table V.) is one of almost complete occlusion of vessels by the invading organism. The degree of bacterial blocking was not as great in test plants 5 and 7; the slower reduction of transpiration rate and the freshness of certain apical leaves in these plants at the close of the experiment reflects the lesser degree of blocking. The eosin and sectional analysis showed that some few vessels in the large vascular bundles escaped invasion and sufficient water was transported from the roots to the apical leaves to keep these turgid. Strong bleeding developed from the roots of these test plants and indicated that where mechanical blocking was not complete the absorbing

TABLE V.—DISTRIBUTION OF BACTERIA AND THE DEGREE OF BACTERIAL BLOCKING AS DETERMINED BY SECTIONING AFTER EOSIN TRANSPORT TEST, EXPT. 1, CORRELATE WITH TABLE I.

	Test Plant.			
	1.	3.	5.	7.
Base of leaves	*a* Vessels of all bundles completely blocked with bacteria *b* Vessels of all bundles completely blocked with bacteria *c* Vessels of all bundles completely blocked with bacteria *d* Vessels of all bundles completely blocked with bacteria *e* Vessels of all bundles completely blocked with bacteria *f* Vessels of all bundles completely blocked with bacteria *g* Vessels of all bundles completely blocked with bacteria *h* Slight invasion of one lateral bundle	*a* All bundles blocked *b* All bundles blocked *c* All bundles blocked *d* All bundles blocked *e* All bundles blocked *f* One lateral and the central bundle completely blocked. Only 2 3 vessels in other laterals blocked *g* All bundles blocked *h* Both laterals blocked; central bundle free *i* Slight invasion of all bundles	*a* Both laterals blocked; central free *b* Both laterals blocked; central free *c* Both laterals blocked; central free *d* All bundles completely blocked *e* Unilateral invasion *f* Heavy invasion, but eosin able to pass *g* Unilateral invasion *h* Unilateral invasion *i* Unilateral invasion	*a* Both laterals blocked; central free *b* Both laterals blocked; central free *c* All bundles blocked *d* Unilateral invasion *e* All bundles blocked *f* Bacteria present but not in blocking numbers *g* Heavy unilateral invasion *h* Slight bilateral invasion *i* Slight invasion *j* Slight invasion *k* Slight invasion
Stem	Section cut at level of *d* showed complete blocking of two large bundles and of three small bundles. In the third bundle only a few vessels showed eosin	Section at level of *c* showed two large bundles and the three small ones completely blocked. Sections at level of *e* and *h* showed same degree of blocking	Section at level of *d* showed eosin present in 5 6 vessels of each bundle, the rest being blocked	Section at *e* showed bacteria blocking all the earlier formed vessels. Vessels nearer the cambium showing eosin. Section at *j* showed bacteria in one large bundle
Root	Slight watery exudate. Heavy blocking of most vessels	Slight watery exudate. Bacteria present in blocking numbers in most vessels	Strong watery exudate from two bundles. Bacteria present but not in blocking numbers; one bundle completely blocked	Strong watery exudate. Bacteria present but not in blocking numbers

TABLE VI.—DEGREE OF BACTERIAL BLOCKING AS DETERMINED BY EOSIN TEST AND SECTIONING AT CLOSE OF TRANSPIRATION EXPT. 4.

Test Plant Number.	4.	8.	9.	10.
Leaves..	*a*, *b*, *c* All bundles blocked and considerable breakdown of xylem *d* Both laterals blocked; central free *e* One lateral and central bundle blocked; lighter invasion in other lateral *f* One lateral and central bundle blocked; lighter invasion in other lateral *g* One lateral and central blocked; eosin in other lateral *h* Eosin in all bundles Bacteria in vessels of one lateral *i* One lateral and central blocked *j* Central bundle only blocked, eosin in laterals	*a*, *b*, *c*, *d* All bundles blocked by bacteria *e* Both laterals and central bundles invaded, but eosin was able to pass *f* All bundles completely blocked *g* Half the vessels in one lateral blocked. Rest of vessels in this bundle, and vessels in other bundles passed eosin *h* Slight invasion of all bundles *i* One lateral heavily invaded; other lateral free of bacteria but no eosin passing due to blocking lower down in the stem	*a*, *b*, *c*, *d*, *e* Complete blocking of vessels *f* Only 2 3 vessels invaded in one lateral; all other vessels showing eosin *g* Both laterals and central blocked *h* One lateral blocked, other lateral free of bacteria and showing eosin *i* One lateral and central bundle blocked; other lateral only partially blocked *j*, *k* All bundles blocked *l*, *m* One lateral and central bundle blocked	*a*, *b*, *c*, *d*, *e* All bundles completely blocked *f* One lateral blocked *g* All bundles blocked *h* One lateral and the central bundle blocked *j*, *k*, *l*, *m* Fairly heavy invasion
Stem ..	Section at level of *d* showed one large bundle quite blocked. A second large bundle was heavily invaded and the third only slightly	Level of *d*. A few vessels of each bundle allowed passage of eosin, the rest being blocked by bacteria	Level of *g*. Eosin was present only in one large bundle, the others being blocked	Eosin passing in a few vessels of one large bundle at level of *f*
Root ..	Not recorded ..	Not recorded ..	Bacteria heavily invading vessels down to 2 inches below soil level	Bacteria filling approximately 50 per cent. of larger vessels

mechanism was not affected by the presence of bacteria or their metabolic products. An illustration of the degree of blocking commonly recorded when the statement "all bundles completely blocked" is made in the tables, may be seen in Pl. XI., fig. 3. The typical degree of blocking associated with epinastic response of leaves is illustrated in earlier papers (Grieve, 1936, 1939). The details given in the 1939 paper apply as well to the physiology of wilting although that aspect of interpretation was not there stressed. The data presented above is considered to provide conclusive evidence for the mechanical blocking theory in the case of tomato and potato plants.

It is of value here to consider results and interpretations of other workers. Van der Meer (1929), while not using the refinements of technique here employed in assessing the value of blocking, nevertheless made many careful observations. She

employed an eosin transport test followed by "barking" of the stem to observe the movement of eosin, and sectioning to determine the presence or absence of bacteria. Unfortunately her observations on the presence or absence of bacteria at the bases of leaves are limited to +, ++ or — signs, no clear indication of numbers present being given. Furthermore no information is contained in her tables on the occurrence of bacteria in the stem; consequently it is not possible to determine any relation between the bacteria in the vessels, the transport of eosin and the wilting of leaves in the tomato plants she used. Conclusions reached by Van der Meer, however, are as follows:—(*a*) Leaves can be turgid, although *B. solanacearum* be present in petiole and lamina, (*b*) flaccid leaves generally contain the parasite, in some cases, however, they are present only in small numbers, (*c*) sometimes loss of turgidity is not accompanied by the presence of *B. solanacearum*. Her interpretation of these results is that the invading organism did not cause the symptoms in a "direct way," which led her to include gum formation as a factor in the induction of wilting. It is not disputed that the conditions she lists do occur and in fact many similar conditions have been recorded, but all are readily explained without having to invoke any other factor than blocking by bacteria. By the stem sectioning or stem maceration analysis it has been demonstrated that the wilting of leaves in the presence of only small numbers of bacteria or no bacteria at all, is due to invasion lower down in the stem. In an earlier paper (Grieve, 1939) this point was stressed in relation to epinastic response of leaves. Turgidity of leaves in the presence of considerable though not "blocking" numbers of the invader is of common occurrence; epinasty of leaves which frequently precedes wilting, seldom occurs in the absence of the organism. Eosin conduction tests also showed that if even a few vessels remained uninvaded the dye passed upwards in them. It is considered that in the absence of complete section analyses of infected plant stems and roots, Van der Meer was led to an incorrect conclusion in the case of tomato plants. Certain of her own observations contradict her conclusions as she makes the following statements:—"The vessels containing the parasite remain *uncoloured* and the leaves or leaf parts obtaining water from these tracheae remained green," and "the experiments taught that the bacteria in the vessels form column-like masses which lengthen at both sides and branch when lateral ways (leaf petiole or lateral roots) make such possible."

A study of Smith's observations and illustrations (Smith, 1913, 1920), show that in the tomato and potato plants he studied, the invading organism was present in "blocking" numbers at the wilting phase. He, however, expressed no definite opinion as to the cause of wilting. Hutchinson (1913) sponsored the toxin theory of wilting in the case of tobacco plants and described an experiment which he considered proved that mechanical bacterial

blocking could not be responsible. He made a cut half way through the stem of a tobacco plant and inserted a thin strip of plasticene, believing that this would approximate to mechanical blocking in the vessels. No wilting occurred in the leaves above the cut and as he considered that the interference with water supply was greater here than in the case of a bacterially invaded stem, concluded that some other factor must cause wilting of the leaves. Hutchinson's experiment was repeated and others devised to check his interpretation. It was first shown that his expectation of wilting in leaves above a cut blocked with plasticene was not in accordance with water movement in the plant. On placing a plant with its stem locally blocked in the manner he described and with its cut root in eosin solution, the dye passed up the uninterrupted bundles to the apex and then down the far side to the region blocked by the plasticene, passing into all the leaves on the way. This indicated that the localized blocking had caused no real interference with the movement of water and made it clear why no wilting occurred.

Critical experiments were next carried out to test under what conditions of artificial blocking, wilting of leaves in tomato and potato plants would occur. The position of the vascular bundles in the stems of these plants is easily seen when they are placed in front of a bright light, and the procedure was adopted of cutting out very small pieces of the vascular bundles in the stem, just below or above a leaf, in order to interrupt the continuity of the water stream there. Vaseline was used to block the cut regions. Experimental results obtained were as follows:—When one lateral bundle leading to a leaf was cut through and blocked, the leaf remained fresh; when a lateral and a central bundle were cut the leaf still remained fresh, but when in addition the terminal leaflet of the leaf being experimented with was removed, distinct unilateral flaccidity resulted. The removal of the terminal leaflet when one lateral and the central bundle to the leaf are interrupted, prevents the return flow of water from the other uninjured lateral bundle. Again, when in addition to the above treatments the vascular bundle leading to the next leaf above was cut, flaccidity became much more pronounced. On cutting all bundles leading up to a leaf together with the bundle leading to leaves higher on the stem, severe wilting of this leaf occurred in one hour. In certain experiments where only slight flaccidity of leaflets of a leaf occurred after cutting selected bundles, transport of dye showed that not all the vessels of the bundles had been cut and these few intact ones were transporting water. From this it was evident that even a few intact vessels could supply the necessary water to maintain a degree of turgidity in the leaf. The results of these experiments may be correlated with observations on actual occurrences in infected plants; thus the cutting of one lateral leaf bundle fails to induce wilting and is paralleled by the

fact that invasion of one lateral bundle fails to cause wilting, until bacteria in "blocking" numbers grow so far out in the lamina that water cannot be conducted back from the opposite side. Bacterial blocking of two laterals approximated to the cutting of two laterals and of the vascular bundle leading to the second leaf above. The fact that a few intact vessels in a bundle could allow conduction of sufficient water to maintain turgidity in leaves, explains in large measure why leaves of infected plants can remain turgid so long when bacterial invasion is fairly heavy. Not all vessels are blocked, except in the later stages of the disease and the few free vessels can pass sufficient water to keep the leaves reasonably fresh. This turgidity of leaves is not to be confused, however, with the effect of the numbers of bacteria on the rate of transpiration. The volume of water required to keep a leaf of tomato or potato plant turgid under average glasshouse conditions is relatively small (cf. Knight, 1922), while as experiments on companion leaves proved, transpiration may be greatly reduced while leaves still appear quite fresh.

In dealing with the general subject of wilting in plants, Knight (1922) suggested that in the summer, due to a variety of factors, such as increase of atmospheric evaporating power and decrease in soil moisture, a greater tension was placed upon the water columns in the vessels leading to the gradual replacement of water by air, the water columns being severed one by one with the increasing tension until the number remaining unbroken could not supply the leaves with sufficient water to keep the plant alive. The correctness of this conclusion was challenged by Bode (1923) who contended that even in the very last stages of wilting there were no air bubbles in the xylem.

For a clearer understanding of the mechanism of wilting in infected plants these two viewpoints were tested.

Bode's technique was used to determine whether air bubbles developed in vascular bundles of tomato and potato plants showing (*a*) artificially induced wilting due to drying out of the soil, and (*b*) bacterially induced wilting. No bubbles were found in the vessels of tomato plants showing severe wilting due to drying out of the soil so that confirmation of Bode's work was obtained. The examination of bundles of infected plants to see the action of the bacteria proved somewhat more difficult as it was necessary to find the apices of the bacterial masses and to determine whether the water columns were intact above them. The picture finally found was as follows:—Organisms at the head of the growing masses in the vessels were rapidly dividing and were actively motile. The head of the bacterial column advanced due to packing of the organisms. No air bubbles were observed above the bacterial masses in individual vessels. Eosin dye failed to pass upwards through the bacterial mass, but after a longer period dye was observed coming downwards in the vessels above the

bacterial mass having first passed up the free vessels in other bundles. This observation indicated the continuity of the water columns above the bacteria. As stated above no eosin passed from below through the bacterial mass, but the possibility remains that a very small but sufficient amount of water was passing between the packed bacteria to keep the water column intact, but this volume of water was quite insufficient to keep the leaves turgid. These experiments offer no confirmation of the view that actual rupturing of the water columns occurs, rather the reverse because if it were so the bacteria could not grow upward and downwards in the vessels.

Bacterial action in the vessels may be pictured as follows:— The growing bacteria first fill one vessel and interrupt gradually the water flow in it until finally complete blocking occurs, then they spread to a second and repeat the process, and so on, finally few or no vessels in a bundle or bundles leading to one or more leaves are left uninvaded and blocked. Wilting then occurs. The process in the earlier stages is localized and the interruption of the water supply relatively slow. Wilting gradually becomes systemic as the bacteria debouch into and block new vessel groups at the points of junction of bundles in the stem.

Summary.

1. The effect of *Bacterium solanacearum* on the water relations of tomato and potato plants has been analysed in relation to speed of invasion and the production of leaf epinasty and wilting.

2. It has been shown that the march of transpiration in infected and control plants runs parallel until several leaves of the infected plants are showing epinasty and unilateral or bilateral wilting. As increasing numbers of leaves are affected a gradual depression of transpiration rate occurs. The maintenance of a high rate of transpiration in infected plants despite a considerable reduction in effective leaf area in the earlier stages of wilting, was paralleled in experiments on healthy plants in which successive leaves were vaselined. The significance of these results is discussed.

3. The march of absorption in relation to invasion follows a closely similar path to that of transpiration under the same conditions. Where the parasite was inoculated at the stem apex, no reduction in absorption occurred before the bacteria were present in "blocking" numbers in several root vessels after growing downward through the vessels of the stem.

4. The transpiration and absorption experiments, together with eosin transport tests and histological studies, showed in the case of infected tomato and potato plants, that the wilting of leaves was due to gradual mechanical blocking of the vessels by the parasite and not to the presence of tyloses, gum or toxins.

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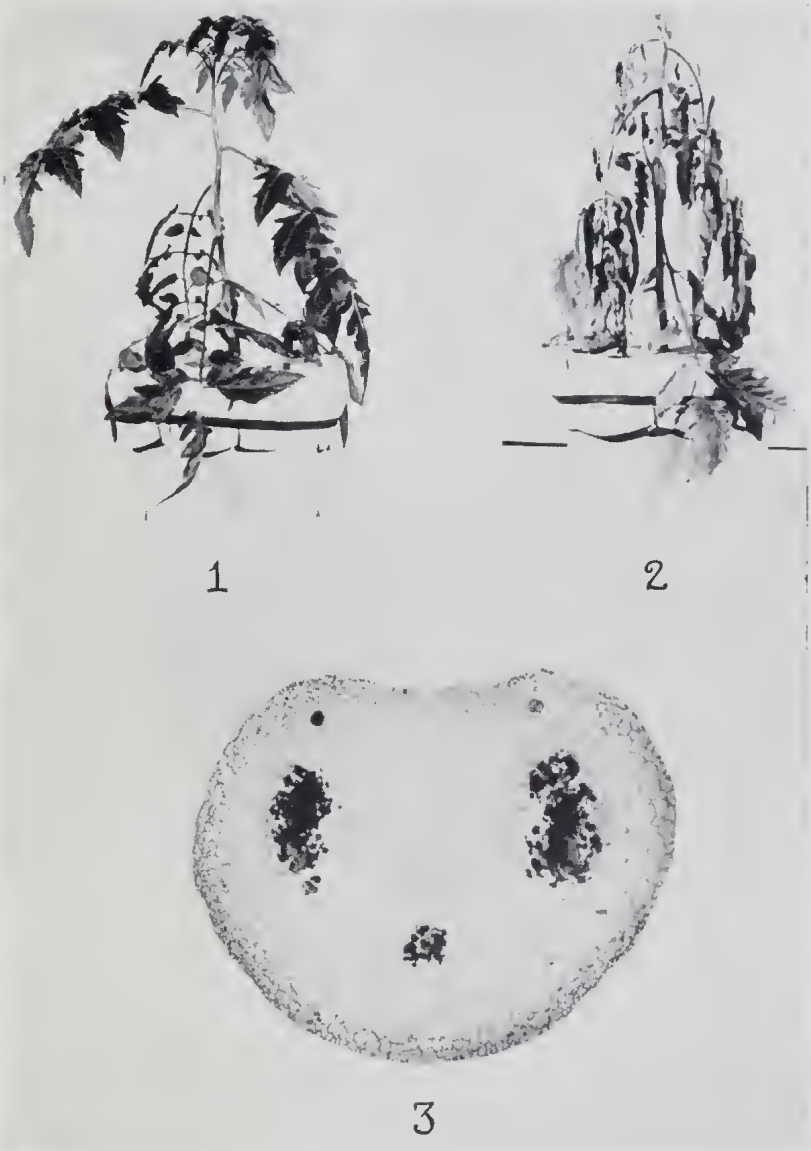
Explanation of Plate.

Plate XI.

Fig. 1. Leaf epinasty preceding bacterial wilting. No obvious depression of transpiration is occurring at this phase. Expt. 4.

Fig. 2. Wilting due to bacterial invasion. This is the same plant as in Fig. 1, photographed at the close of Expt. 4.

Fig. 3.—Photograph of a transverse section at the base of a petiole of a wilted leaf. Note the complete blocking of vessels by bacteria. Breakdown of xylem is also occurring.



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